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Natural Abundance 13C NMR Spectroscopy of Double-Stranded DNA

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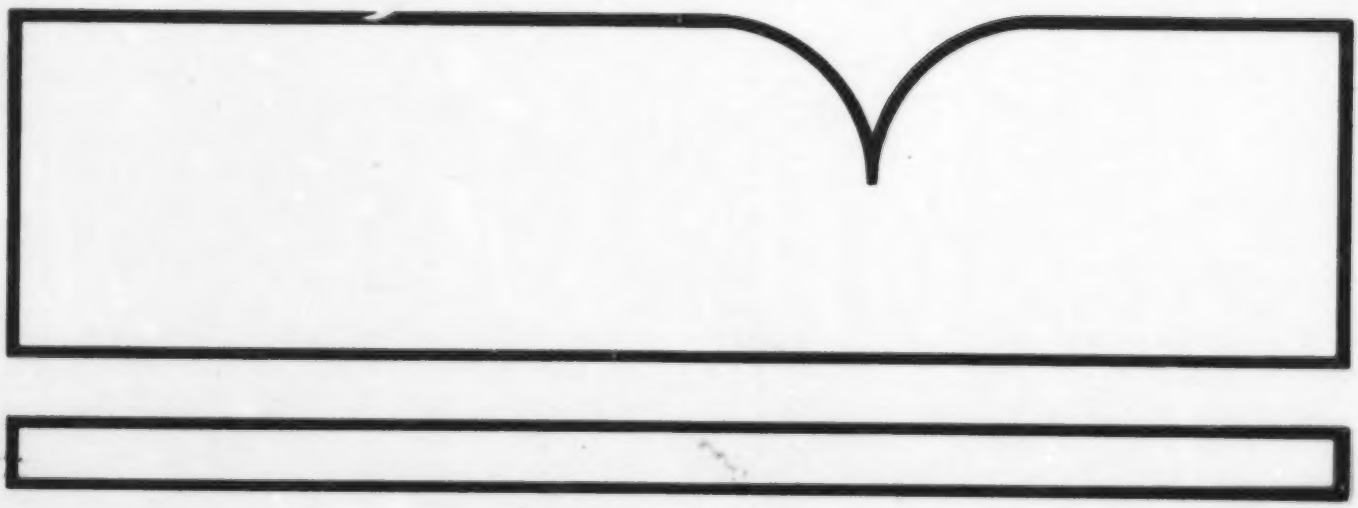

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16. ABSTRACT

Although ^{13}C NMR spectroscopy has already proved extremely useful in studies of biopolymers, including t-RNA's, and single-stranded polynucleotides, no successful study of native double-stranded DNA has been reported. This failure is mainly due to extremely unfavorable ^{13}C spin relaxation parameters (very short T_2's and long T_1's) expected for the long and relatively rigid chains of typical DNA preparations.

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Natural Abundance ^{13}C NMR Spectroscopy of Double-Stranded DNA

Sir:

Although ^{13}C NMR spectroscopy has already proved extremely useful in studies of biopolymers,[1] including t-RNA's,[2] and single-stranded polynucleotides,[3] no successful study of native double-stranded DNA has been reported.[4] This failure is mainly due to extremely unfavorable ^{13}C spin relaxation parameters (very short T_2's and long T_1's) expected for the long and relatively rigid chains of typical DNA preparations (length >10 000 Å; weight $>2 \times 10^6$ Daltons).

Standard hydrodynamic calculations of rotational correlation times of rigid rod models suggested that the spectrum of short DNA molecules, on the order of 100 nucleotide pairs (NP), could be observed with modern instrumentation. DNA near this size can be obtained from native chromatin digested extensively with micrococcal nuclease. Histone binding to DNA in chromatin restricts access of the nuclease to specific sites. As a result, digestion yields DNA that is mostly near 140 NP in length along with some smaller fragments.[5,6] Unlike sonication, this well-studied method of reducing the size of DNA is very gentle and yields fragments that are almost entirely doubly stranded, with few unpaired nucleotides extending from the ends.[7]

Table I

	1,[a,b] heat denatured DNA (52 mg/5 mL), 67.9 MHz[f]		2,[a,c] native DNA (117 mg/3.5 mL), 67.9 MHz		3,[a,d] native DNA (171 mg/3.5 mL), 67.9 MHz		4,[a,d] native DNA (87 mg/2.5 mL), 37.7 MHz		5,[a,d,e] ethidium bromide + native DNA (81 mg/2.5 mL), 37.7 MHz	
	ppm	$\Delta\nu_{1/2}$, Hz	ppm	$\Delta\nu_{1/2}$, Hz	ppm	$\Delta\nu_{1/2}$, Hz	ppm	$\Delta\nu_{1/2}$, Hz	ppm	$\Delta\nu_{1/2}$, Hz
					Pentose Carbons					
C-2′	38	114	37.5	243	37.0	196	37.5	150	40.5	338
C-3′	77	59	75.0	245	74.5	295[g]	76.5	165	76.0	
C-5′	66.5	64	64.5	117	64–70[g]		65.0	65	64.0	364
					Base Carbons					
T(Me)			12.0	117	12.0	137	12.5	60	15.0	234
T-5			110.5	176	110.5	114	111.0	120		
G-5			114.5	137	114.5	98	115.0	120		
A-5			116.5	125	116.5	82	117.5	105		

[a] Sample number. [b] Conditions: 90 °C, 0.1 M NaCl (pH 5.62). [c] Conditions: ambient temperature, 0.1 M NaCl (pH 5.62). [d] Conditions: ambient temperature, 42.2 mM Na_2PO_4, 15.6 mM $NaHPO_4$ (pH 7.35). [e] (Ethidium bromide)/(DNA phosphate) = 1:8. [f] ^{13}C frequency. [g] This peak is composed of several lines which may be noise.

Accordingly, for this study we isolated a large quantity of DNA from calf thymus chromatin digested to the limit, when the chromatin precipitates and ~50% of the DNA is rendered acid soluble.[8] Chromatography of this DNA on Bio-gel A0.5m (Bio-Rad Laboratories)[9] yielded fragments ranging from 110 to 170 NP long, with a median length of 140–145 NP.[10] Further treatment with micrococcal nuclease under conditions where it acts almost solely on single-stranded DNA[11] did not affect the electrophoretic mobility of the major band. The single-stranded DNA contents of preparations used for NMR studies were assessed from measurements of hyperchromicity upon thermal denaturation, and also from release of acid-soluble oligonucleotides upon treatment with single-strand specific nuclease S1[12] (Sigma). Results from both methods were in close agreement and indicated single-strand contents of 3–6% for the samples *after* NMR analyses. (The higher value probably is overestimated since a slow, nonspecific nuclease activity was noted in the S1 preparation.) The integrated ^{13}C spectra do not allow for major contributions from single-stranded DNA.

Two superconducting solenoid-based spectrometers were used to acquire ^{13}C NMR spectra at 67.9 and 37.7 MHz. High-field spectra were taken on a Bruker HX-270 and intermediate field spectra on the SEMINOLE spectrometer[13] equipped with a broad-band side-spinner design probe.[14] A sample spectrum of native DNA is shown in Figure 1. Table I summarizes data from five spectra: three of native DNA, one of heat denatured DNA, and one of native DNA containing intercalated ethidium bromide.

Quantitative interpretations of these preliminary spectra were not attempted, but several trends deserve comment. First, the assigned resonances of native DNA are quite close to literature values for mono- and oligonucleotides.[15-17] For example, C-1′ and C-5′ resonances are within 1.5 ppm of the positions reported for deoxyribonucleotides. C-2′ is shielded by ~2.5 ppm, while C-4′ and C-3′ are deshielded by 2–3 ppm relative to the mononucleotide resonances. Changes in shieldings of 0.4–3.4 ppm were observed for the base ring carbons.

The observed line widths for native ~140-NP DNA are also interesting. In general these are factors of 5 to 10 *less* than the line widths predicted from the rotational correlation time of a rigid rod, suggesting that internal torsional or librational motions contribute significantly to the spin relaxation. The nonprotonated ring carbon resonance line widths at each magnetic field (frequency) are nearly comparable with the sugar protonated-carbon line widths, whereas, in ^{13}C spectra of proteins, nonprotonated carbon lines are much narrower

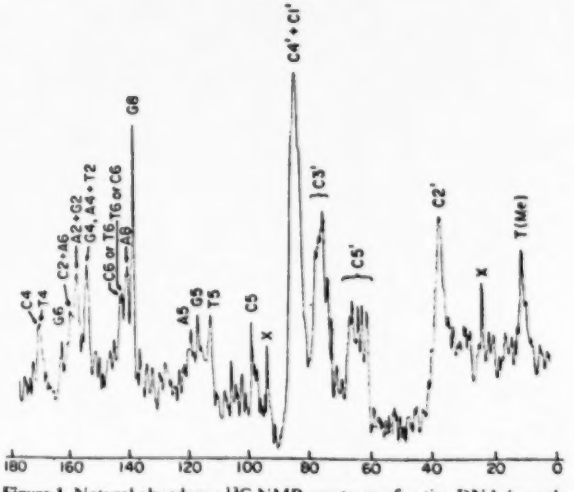

Figure 1. Natural abundance ^{13}C NMR spectrum of native DNA (sample 3 in Table I) at 67.9 MHz; 43 000 scans were accumulated with a cycle repeat time of 1.5 s. Chemical shifts are reported in parts per million relative to an external Me_4Si standard.

than CH lines (lower efficiency dipolar relaxation).[18] The relatively narrow sugar CH and CH_2 lines reflect additional internal motions, especially at C-5′. This flexibility seems restricted in the DNA–ethidium complex. The field dependence of several of the CH and nonprotonated carbon line widths is inverted, with narrower lines observed at the lower frequency. The narrower lines at 37.7 MHz could be due in part to susceptibility inhomogeneity broadening[19] or to the nature of torsional reorientation of C–H vectors along the "rigid" DNA rod (20 Å × ~500 Å). Most likely the larger line widths at higher field contain contributions from chemical-shift dispersion (subunits in different chemical environments). This will be investigated further.

Finally, heat denaturation significantly decreased the line widths observed for this short DNA, as expected from an increase in chain conformational flexibility. In contrast, intercalation of ethidium caused a significant increase in line widths, presumably due to an increase in length and/or a decrease in torsional flexibility. These line-width changes are consistent with established hydrodynamic data.[20]

Natural abundance ^{13}C spectroscopy of ~140-NP native DNA is now practical. Isolation of smaller double-stranded DNA molecules by the same methods (albeit in lesser yields),

combined with foreseeable improvements in probe and instrument design, should greatly improve spectral quality and permit careful examination of structural details of DNA alone and in combination with specific chemical agents.

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(9) The DNA was dissolved in 0.195 M NaCl, 5 mM Na_2EDTA, 10 mM Tris, 1 mM cacodylic acid, and 0.02% NaN_3. This DNA was fractionated on a Bio-Gel A0.5 m column (5 × 100 cm) eluted with the above buffer made 34% in sucrose (flow rate, 112 mL/h).
(10) DNA fractions from the column were assayed for size (determined by electrophoresis according to Peacock A. C.; Dingman, C. W. *Biochemistry* **1967**, *6*, 1818), pooled, and dialyzed against 1.0 M NaCl. The DNA was precipitated with 1.5 vol. of isopropyl alcohol. After lyophilization to remove volatile impurities, the sample was dissolved in phosphate buffer or NaCl (Table I).
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